

Applicants: Ilan Sela and Sylvia Zeitoune-Simovich
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In the claims:

Please replace the claims with the listing of claims below.

1. - 26. (cancelled)

27. (currently amended) A method of silencing the expression of a target sequence within the genome of a plant ~~or within the genome of a plant infecting pathogen present in said plant~~, which method comprises the steps of:

- a) providing a first plant capable of regenerating;
- b) hybridizing said first plant with a second plant double transformed with:
 - i) a first DNA construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, said construct further comprising at least one plant promoter and at least one plant terminator sequence operably linked to said T7-pol; and with
 - ii) a second DNA construct comprising a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7 and a 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising additional regulatory elements operably linked to said targeting sequence; and
- c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is silenced.

28. (currently amended) A method of silencing the expression of a target sequence within the genome of a plant ~~or~~

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~~within the genome of a plant infecting pathogen present in said plant prior to the following manipulation~~, which method comprises the steps of:

- a) providing a first plant comprising said target sequence, said plant being capable of regenerating;
- b) hybridizing said first plant with a second plant transformed with a DNA construct comprising the T7 RNA polymerase gene (T7-pol), a NLS sequence, a plant promoter and a plant terminator sequence operably linked to said T7-pol, a T7 promoter (pT7), a targeting sequence downstream to said pT7, and at least one additional promoter sequence operably linked to said targeting sequence; and
- c) selecting those plants obtained by the hybridization of step (b), in which the expression of said target sequence is silenced.

29 - 32. (cancelled)

33. (previously presented) A method for silencing the expression of a target gene within a plant cell comprising the steps of:

- a) transforming a plant cell with a first construct comprising the T7 RNA polymerase gene (T7-pol), a NLS sequence, and at least one promoter and at least one terminator sequence operably linked to said T7-pol;
- b) selecting plant cells transformed with said first DNA construct according to step (a);
- c) transforming the selected plant cells obtained in step (b) with a second DNA construct comprising a T7 promoter sequence, a targeting sequence

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downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;

- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct;

whereby transformation of said plant cell with said first and second DNA constructs renders the expression of said target sequence silenced.

34 - 36. (cancelled)

37. (previously presented) A method for silencing the expression of a target gene within a plant cell comprising the steps of:

- a) transforming said plant cell with a DNA construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, said construct further comprising at least one plant promoter sequence and at least one plant terminator sequence operably linked to said T7 polymerase gene, a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7, and at least one additional terminator sequence operably linked to said targeting sequence, which DNA construct is capable, upon transformation thereof into a plant cell, of rendering the expression of a target sequence in said plant cell silenced; and

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- b) selecting plant cells transformed with said DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant.

38 - 39. (cancelled)

40. (currently amended) A method for silencing the expression of a target gene within a plant comprising the steps of:

- a) transforming a first population of plant cells with a first construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, and further comprising at least one plant promoter and at least one plant terminator sequence operably linked to said T7-pol;
- b) selecting the cells obtained in step (a), cells transformed with said first DNA construct, and regenerating said selected cells to provide a differentiated flowering plant; [[or]]
- c) transforming a second population of plant cells with a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence;
- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct, and regenerating said selected cells to provide a differentiated flowering plant; and

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- e) hybridizing a first plant transformed with said first DNA construct as obtained in (b), with a second plant transformed with said second DNA construct as obtained in (d), thereby providing a double-transformed plant in which the expression of said target gene is silenced.

41. - 43. (cancelled)

44. - 52. (not entered)

53. (new) A method of silencing the expression of a target sequence within the genome of a plant, wherein said target sequence is one of:

- I) a gene which encodes a plant protein or peptide product,
- II) a transcribed non-coding nucleic acid sequence, or
- III) a nucleic acid sequence which is a fragment of (I) or (II),

which method comprises the steps of:

- a) providing a first plant capable of regenerating;
- b) hybridizing said first plant with a second plant double transformed with:
 - i) a first DNA construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, said construct further comprising a p35S plant promoter and a plant terminator sequence, wherein said plant terminator sequence is the NOS terminator or the β -1,3-gluconase terminator, operably linked to said T7-pol; and with

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- ii) a second DNA construct comprising a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7 and a 3' non-translated terminator sequence operably linked to said targeting sequence, wherein said pT7 is the promoter region of the bacteriophage T7 capable of initiating transcription of said downstream targeting sequence and the terminator is the NOS terminator operably linked to said targeting sequence, said construct optionally further comprising additional regulatory elements operably linked to said targeting sequence, wherein said targeting sequence is identical to at least part of a target expressed sequence or is identical to at least part of a non-coding sequence which is a regulatory element sequence and is transcribed; and
- c) selecting those plants obtained by the hybridization of step (b), wherein expression in the progeny of the cross resulting from step (b) is silenced by causing the disappearance of the RNA transcripts of said target sequence.

54. (new) A method of silencing the expression of a target sequence within the genome of a plant, wherein said target sequence is one of:
- I) a gene which encodes a plant protein or peptide product,
 - II) a transcribed non-coding nucleic acid sequence, or

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III) a nucleic acid sequence which is a fragment of (I) or (II),

which method comprises the steps of:

- a) providing a first plant comprising said target sequence, said plant being capable of regenerating;
- b) hybridizing said first plant with a second plant transformed with a DNA construct comprising the T7 RNA polymerase gene (T7-pol), a NLS sequence and further comprising a p35S plant promoter and a plant terminator sequence, wherein said plant terminator sequence is the NOS terminator or the β -1,3-gluconase terminator, operably linked to said T7-pol, a T7 promoter (pT7), a targeting sequence downstream to said pT7, and at least one additional promoter sequence operably linked to said targeting sequence, wherein said targeting sequence is identical to at least part of a target expressed sequence or is identical to at least part of a non-coding sequence which is a regulatory element sequence and is transcribed; and
- c) selecting those plants obtained by the hybridization of step (b), wherein expression in the progeny of the cross resulting from step (b) is silenced by causing the disappearance of the RNA transcripts of said target sequence.

55. (new) A method for silencing the expression of a target gene within a plant cell, wherein said target sequence is one of:

- I) a gene which encodes a plant protein or peptide product,

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- II) a transcribed non-coding nucleic acid sequence, or
- III) a nucleic acid sequence which is a fragment of (I) or (II),

comprising the steps of:

- a) transforming a plant cell with a first construct comprising the T7 RNA polymerase gene (T7-pol), a NLS sequence, further comprising a p35S plant promoter and a plant terminator sequence, wherein said plant terminator sequence is the NOS terminator or the β -1,3-gluconase terminator, operably linked to said T7-pol;
- b) selecting plant cells transformed with said first DNA construct according to step (a);
- c) transforming the selected plant cells obtained in step (b) with a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter, and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, wherein said pT7 is the promoter region of the bacteriophage T7 capable of initiating transcription of said downstream targeting sequence and the terminator is the NOS terminator operably linked to said targeting sequence, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence, and wherein said targeting sequence is identical to at least part of a target expressed sequence or is identical to at least part of a non-coding sequence which is a regulatory element sequence and is transcribed;

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- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct, whereby transformation of said plant cell with said first and second DNA constructs renders the expression of the target sequence in said cell, in a tissue, organ, or plant regenerated from said cell, or in a progeny thereof silenced by causing the disappearance of the RNA transcripts of said target sequence.

56. (new) A method for silencing the expression of a target gene within a plant cell, wherein said target sequence is one of:

- I) a gene which encodes a plant protein or peptide product,
II) a transcribed non-coding nucleic acid sequence, or
III) a nucleic acid sequence which is a fragment of (I) or (II),

comprising the steps of:

- a) transforming said plant cell with a DNA construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, said construct further comprising a p35S plant promoter and a plant terminator sequence, wherein said plant terminator sequence is the NOS terminator or the β -1,3-gluconase terminator, operably linked to said T7-pol gene, a T7 promoter sequence (pT7), a targeting sequence downstream to said pT7, wherein said pT7 is the promoter region of the bacteriophage T7 capable of initiating transcription of said downstream targeting sequence and the terminator is the NOS terminator operably linked to said targeting sequence, and at least one additional

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terminator sequence operably linked to said targeting sequence, and

wherein said targeting sequence is identical to at least part of a target expressed sequence or is identical to at least part of a non-coding sequence which is a regulatory element sequence and is transcribed,

wherein upon introduction of said DNA construct into a cell or plant, the expression of the target sequence in said cell, in a tissue, organ, or plant regenerated from said cell, or in a progeny thereof, is silenced by causing the disappearance of the RNA transcripts of said target sequence; and

- b) selecting plant cells transformed with said DNA construct according to (a) and regenerating said selected cells to provide a differentiated flowering plant.

57. (new) A method for silencing the expression of a target gene within a plant, wherein said target sequence is one of:

- I) a gene which encodes a plant protein or peptide product,
II) a transcribed non-coding nucleic acid sequence, or
III) a nucleic acid sequence which is a fragment of (I) or (II),

comprising the steps of:

- a) transforming a first population of plant cells with a first construct comprising the T7 RNA polymerase gene (T7-pol) and a NLS sequence, and further comprising a p35S plant promoter and a plant terminator sequence, wherein said plant terminator

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sequence is the NOS terminator or the β -1,3-gluconase terminator, operably linked to said T7-pol;

- b) selecting the cells obtained in step (a), cells transformed with said first DNA construct, and regenerating said selected cells to provide a differentiated flowering plant;
- c) transforming a second population of plant cells with a second DNA construct comprising a T7 promoter sequence, a targeting sequence downstream to said T7 promoter and at least one 3' non-translated terminator sequence operably linked to said targeting sequence, wherein said pT7 is the promoter region of the bacteriophage T7 capable of initiating transcription of said downstream targeting sequence and the terminator is the NOS terminator operably linked to said targeting sequence, and wherein said targeting sequence is identical to at least part of a target expressed sequence or is identical to at least part of a non-coding sequence which is a regulatory element sequence and is transcribed, said construct optionally further comprising other additional regulatory elements operably linked to said targeting sequence; and
- d) selecting from the plant cells obtained in step (c), cells transformed with said second DNA construct, and regenerating said selected cells to provide a differentiated flowering plant;
- e) hybridizing a first plant transformed with said first DNA construct as obtained in (b), with a second plant transformed with said second DNA

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construct as obtained in (d), thereby providing a double-transformed plant in which the expression of said target gene is silenced by causing the disappearance of the RNA transcripts of said target sequence.